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## Experimental model for acute kidney injury caused by uropathogenic *Escherichia coli*

### Eksperymentalny model ostrego uszkodzenia nerek wywołany przez uropatogenną pałeczkę okrężnicy

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## Summary

#### Introduction:

Acute kidney injury (AKI) is the rapid deterioration of renal function, diagnosed on the basis of an increase in serum creatinine and abnormal urinary parameters. AKI is associated with increased risk of mortality or chronic kidney disease (CKD).

The aim of the study was to develop an experimental model for AKI resulting from *Escherichia coli*-induced pyelonephritis. *E. coli* was isolated from a patient with clinical symptoms of urinary tract infection (UTI).

#### Material/Methods:

The study included three groups of female Wistar rats (groups 1, 2 and 3), in which pyelonephritis was induced by transurethral inoculation with highly virulent *E. coli* ( $10^5$ ,  $10^7$  and  $10^9$  cfu/ml, respectively). Urine and blood samples for analysis were obtained prior to the inoculation (day 0), as well as 7, 14 and 21 days thereafter.

#### Results:

Aside from a microbiological examination of urine samples, daily urine output, serum creatinine (CreaS), creatinine clearance (CrCl), interleukin 6 (IL-6), fractional excretion of sodium (FENa) and fractional excretion of urea (FEUrea) were determined. A histopathological examination of kidney and urinary bladder specimens was conducted as well. While UTI-related pyelonephritis developed irrespective of *E. coli* inoculum size, AKI was observed only following transurethral administration of *E. coli* at the intermediate and high dose, i.e.  $10^7$  and  $10^9$  cfu/ml, respectively (group 2 and 3).

#### Discussion:

An increase in CreaS and abnormal diuresis were accompanied by changes in parameters specific for various forms of AKI, i.e. FENa and FEUrea. Based on these changes, administration of *E. coli* at  $10^7$  cfu/ml was demonstrated to induce renal AKI, whereas inoculation with  $10^9$  cfu/ml seemed to cause not only ascending pyelonephritis, but perhaps also bacteremia and urosepsis (prerenal component of AKI).

#### Keywords:

experimental model • acute kidney injury (AKI) • pyelonephritis • *Escherichia coli* • rat

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**List of abbreviations:** **AKI** – acute kidney injury, **cfu/ml** – colony forming units/milliliter, **CKD** – chronic kidney disease, **CreaS** – serum creatinine, **E. coli** – *Escherichia coli*, **FENa** – fractional excretion of sodium, **FEUrea** – fractional excretion of urea, **GFR** – glomerular filtration rate, **IL-6** – interleukin 6, **KDIGO** – Kidney Disease Improving Global Outcomes, **NO** – nitric oxide, **UTI** – urinary tract infection.

## INTRODUCTION

Acute kidney injury (AKI) is a clinical syndrome characterized by the rapid impairment of renal functions manifesting themselves as an increase in serum creatinine and urination disorders, classified on the basis of the Kidney Disease Improving Global Outcomes (KDIGO) criteria [36]. AKI may develop as a consequence of various renal diseases, plausibly due to the impaired perfusion of the kidneys induced by a decrease in circulating blood volume, toxic damage or urinary obstruction [27].

Renal causes of AKI include also tubulointerstitial diseases, such as pyelonephritis. The latter is typically a consequence of a bacterial infection. The risk of pyelonephritis-related AKI increases whenever the course of the underlying infection is complicated, mainly due to anatomic abnormalities of the urinary tract, long-term catheterization, immunosuppression, pregnancy or absence of one kidney [24,37]. According to literature, AKI may develop in ca. 2-3% of adults with complicated pyelonephritis [14].

An increase in creatinine levels and urinary disorders observed during the course of pyelonephritis-related AKI result from impaired renal perfusion. The latter is mediated by pro-inflammatory cytokines released by white blood cells accumulated at the site of the inflammation. A decrease in glomerular filtration rate (GFR) causes alterations of the tubules, such as the destruction of actin cytoskeleton, epithelial intercellular relaxation, impaired function of integrins and epithelial exfoliation into the tubular lumen [28,34]. Clogging of tubules by uromodulin casts and exfoliated epithelial cells results in an increase in intratubular hydrostatic pressure. Renal ischemia is further enhanced due to a predominance of locally synthesized vasopressors, such as endothelin and thromboxanes, over vasodilators, e.g. nitric oxide (NO) and prostacyclin [23]. Regeneration of renal tissue is a long-term process that may eventually lead to the total restoration of kidney function; nevertheless, in nearly a half of the cases, AKI results in permanent renal impairment [1,38,41].

In previous experimental studies, ascending AKI resulting from pyelonephritis has been induced by intrabladder (laparotomic) [3,12] or transurethral [9,18] administration of an etiological factor (fungus, bacte-

rium), its direct delivery to renal parenchyma [2,7,40] or intravenous injection [35,39]. The transurethral model for pyelonephritis induction accurately mimics pathogenesis of UTI in humans. Aside from anesthesia, the only invasive procedure is urinary catheterization, which minimizes the risk of complications. Minimal invasiveness of the procedure and the lack of organ damage result in relatively low morbidity.

The aims of the study were:

- to develop an experimental model for ascending AKI being a consequence of pyelonephritis caused by infection with *E. coli*, the most common organism isolated from patients with bacterial UTI;
- to analyze the relationship between the inoculum of *E. coli* used to induce pyelonephritis, the course of infection and the degree of kidney damage.

## MATERIALS AND METHODS

### Animals.

The study included 30 female Wistar rats 10 weeks of age and ca. 200 g body weight. The animals were kept under a 12 h day-to-night cycle with unlimited access to water and food (Labofeed, Kcynia, Poland). All experimental procedures were approved by the Ethics Committee for Experiments on Animals at the Jagiellonian University in Cracow (decision no. 133/2012).

### Experimental groups.

The rats were divided into three groups, 10 animals each. Depending on a group, pyelonephritis was induced by transurethral inoculation with 500 µl of suspension containing 10<sup>5</sup> (group 1), 10<sup>7</sup> (group 2) or 10<sup>9</sup> cfu/ml (group 3) of *E. coli*. The procedure was conducted under general anesthesia (pentobarbital, Morbital, Biowet Puławy, Poland, 20 mg/kg intraperitoneally).

Urine and blood parameters determined prior to inoculation with the bacteria (day 0) constituted the reference value for further measurements. Since no significant intergroup differences were found at day 0, the results were pooled and the mean values for all 30 animals were considered as the baseline. This enabled us to apply the

3R principle and to reduce the number of examined animals due to a more accurate selection of statistical methods [31].

## Bacteria.

*E. coli* used in the experiment originated from a patient with acute pyelonephritis. The strain expressed genes for several virulence factors, such as FimH, papC and *sfaD/E* for type 1 adhesin, type P and S fimbriae, respectively. Furthermore, the isolate tested positively for *iroN* gene encoding a receptor for iron ions, *salmochelin*, as well as for *cnf-1* gene encoding cytotoxins, among them tumor necrosis factor type 1 (TNF1).

## Sampling and analysis of the material.

Prior to the induction of bacterial pyelonephritis (day 0), as well as 7, 14 and 21 days thereafter, blood samples for biochemical analyses were obtained from the tail vein, and urine samples were collected for microbiological examination, determination of biochemical parameters (creatinine, sodium, urea, IL-6) and measurement of 24-h urine output (metabolic cage for rats, 150 - 300g, Tecniplast, Italy).

Furthermore, 24-h ingestion of water was determined at days 0, 7, 14 and 21, along with body temperature the studied rats (digital rectal thermometer for rodents, Vivari, UK).

During the course of microbiological examination, urine, in 1- $\mu$ l aliquots, was cultured onto appropriate differentiating media: Columbia Lab-Agar Base (Biocorp) with 5% sheep blood for the isolation of microorganisms with different growth requirements (aerobic Gram-positive and Gram-negative bacteria), and Mac Conkey Lab-Agar (Biocorp) for the isolation of *Enterobacteriaceae* (Gram-negative bacilli). The dishes were incubated for 24 h at 37°C under aerobic conditions. Then, growing colonies were identified and counted; bacterial counts were expressed as cfu per 1 ml of urine.

Concentrations of sodium in blood and urine were determined with the aid of a flame photometer (Corning 450). Blood and urinary concentrations of creatinine were measured with Cobas 6000 analyzer, using C501 biochemical module (Roche). Urinary IL-6 was determined

by means of ELISA, with Rat IL-6 ELISA KIT (RayBiotech®, USA).

On the 21<sup>st</sup> day of the experiment, the animals were killed by a pentobarbital overdose (Morbital, Biowet Puławy, Poland, 100 mg/kg intraperitoneally). Then kidney and urinary bladder specimens for histopathological examination were obtained, washed in saline, and fixed for 24 h in 8% formalin solution in phosphate buffer (PBS, pH 7.4). Subsequently, the tissue was washed with running water for 2 h and dehydrated in ascending ethanol gradient (50-100%). Before being embedded in paraffin, the specimens were treated with xylene, and then incubated for 2 h at 37°C with xylene and paraffin mixture in 1:1 ratio. Subsequently, the material was treated twice with pure paraffin (2 h at 62°C), embedded in paraffin blocks, cut with a microtome, mounted onto glass slides and dried in an incubator at 37°C. The severity of inflammation was assessed in 10 consecutive fields of hematoxylin-eosin (HE)-stained slides, using an AXIOPHOT light microscope (Zeiss, Germany). During the microscopic analysis, the location and characteristics of neutrophil and mononuclear cell infiltrate were determined within the kidney parenchyma and urinary bladder wall.

## Statistical analysis.

Statistical analysis was conducted with Statistica 8 (StatSoft). The results for each group, presented in the form of tables and graphs, were expressed as a mean and standard deviation. Pooled results for all animals, determined prior to bacterial inoculation, were considered as the baseline for all analyses. Intergroup comparisons were conducted with Fisher's ANOVA and HSD Tukey test. The differences were considered significant at  $p \leq 0.05$ .

## RESULTS

### Microbiological examination of urine

Following the induction of pyelonephritis, all animals, irrespective of the group and experiment stage, presented with bacteriuria. No association was found between the size of *E. coli* inoculum and the amount of bacteria recovered from urine, and the analyzed groups did not differ significantly in terms of their urinary bacterial counts (Table 1).

**Table 1.** The microbiological examination of urine (the result given in cfu/ml)

Day	Group 1 10 <sup>5</sup> cfu/ml	Group 2 10 <sup>7</sup> cfu/ml	Group 3 10 <sup>9</sup> cfu/ml	ANOVA
0	<10 <sup>1</sup>	<10 <sup>1</sup>	<10 <sup>1</sup>	
7	2x10 <sup>6</sup> ±5x10 <sup>6</sup>	4.8x10 <sup>4</sup> ±7,1x10 <sup>4</sup>	13.5x10 <sup>4</sup> ±14,5x10 <sup>4</sup>	NS
14	2x10 <sup>6</sup> ±5x10 <sup>6</sup>	3.8x10 <sup>4</sup> ±2,9x10 <sup>4</sup>	2.5x10 <sup>6</sup> ±3,8x10 <sup>6</sup>	NS
21	9x10 <sup>4</sup> ±1x10 <sup>5</sup>	5.3x10 <sup>4</sup> ±1,5x10 <sup>4</sup>	3.5x10 <sup>6</sup> ±8x10 <sup>6</sup>	NS
NS – not statistically significant				

### Histopathological analysis of kidney specimens

Animals from group 1 presented with chronic pyelonephritis and tubulointerstitial nephritis, manifesting as a presence of low-grade lymphocytic infiltration. Inflammation was limited to the renal medulla.

In specimens from group 2, chronic pyelonephritis was found, along with ulceration and tubulointerstitial nephritis with massive lymphocytic and granulocytic infiltration of both renal pelvis and medulla. Similar to group 1, however, the inflammation did not extend beyond the medulla.

In rats from group 3, histopathologic examination revealed chronic pyelonephritis and tubulointerstitial nephritis involving both medulla and cortex of the kidneys, and characterized by massive lymphocytic and granulocytic infiltration.

### 24 h urine collection

At 14 and 21 days after induction of pyelonephritis, all rats presented with lower 24 h urine output than at the baseline. While at day 14, 24 h urine output in all experimental groups was significantly lower than at the baseline. At day 21, urinary excretion in group 1 was similar to that observed prior to the bacterial inoculation, and the only significant differences referred to groups 2 and 3.

Moreover, significant differences were found in 24 h urine output of animals from groups 1, 2 and 3 (Figure 1).

### CreaS

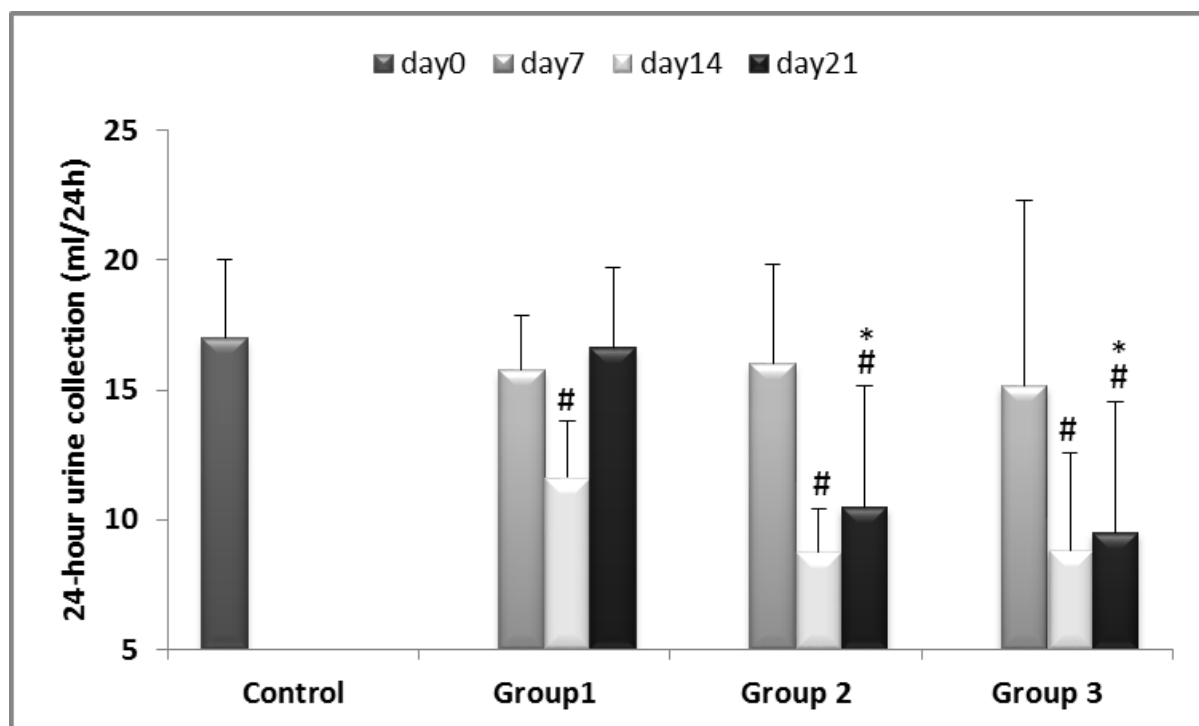
An increase in CreaS, corresponding to the deterioration of renal function, was observed as early as at day 7. In group 3, CreaS was significantly higher than at the baseline beginning at day 7, and in group 2 starting at day 14. Moreover, significant differences were observed in CreaS of animals from group 1 and 3 (Figure 2).

### CrCl

Animals from group 3 presented with significantly lower CrCl values than at the baseline at days 7 and 14, and rats from group 2 no earlier than at day 14. Moreover, significant differences were found in CrCl of rats from experimental groups 1, 2 and 3 (Table 2).

### FENa

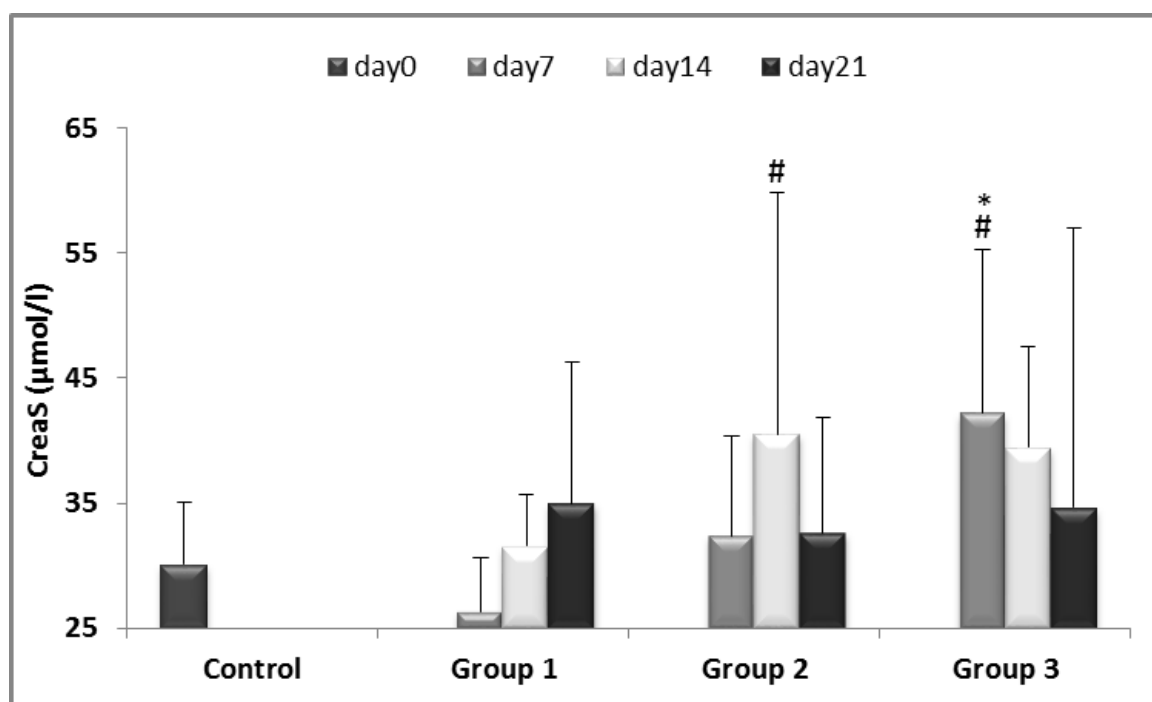
In animals from group 1, FENa determined at day 7 was significantly lower than at the baseline. A similar phenomenon was also documented in group 3, but the significant decrease in FENa was observed not only at day 7, but also at day 21. In contrast, in animals from group 2, FENa determined at the 14<sup>th</sup> day of the experiment was significantly higher than the baseline. Furthermore,



**Fig. 1.** 24-hour urine collection (the result given in ml/24h)

# - statistically significant differences between the study group and the control group ( $p \leq 0.05$ )

\* - statistically significant differences between the study group and the group 1 on the corresponding day ( $p \leq 0.05$ )



**Fig. 2.** CreaS (the result given in  $\mu\text{mol/l}$ )

# - statistically significant differences between the study group and the control group ( $p \leq 0.05$ )

\* - statistically significant differences between the study group and the group 1 on the corresponding day ( $p \leq 0.05$ )

**Table 2.** ClCr (the result given in ml/min)

Day	Group 1. $10^5 \text{cfu/ml}$	Group 2. $10^7 \text{cfu/ml}$	Group 3. $10^9 \text{cfu/ml}$	ANOVA
0	$1.36 \pm 0.42$	$1.36 \pm 0.42$	$1.36 \pm 0.42$	
7	$1.72 \pm 0.61$	$0.92 \pm 0.25$ * $p=0.003$	$0.77 \pm 0.22$ # $p=0.023$ * $p=0.001$	$p < 0.001$
14	$1.1 \pm 0.31$	$0.7 \pm 0.34$ # $p=0.001$	$0.66 \pm 0.19$ # $p=0.001$	$p < 0.001$
21	$1.27 \pm 0.4$	$0.93 \pm 0.33$	$0.99 \pm 0.42$	NS

NS – not statistically significant

# - statistically significant differences between the study group and the control group – HSD Tukey Test

\* - statistically significant differences between the study group and the group 1 on the corresponding day – HSD Tukey Test

statistically significant differences in FENa were found between experimental groups 1, 2 and 3 (Table 3).

### FEUrea

At day 14, FEUrea in animals from group 3 was lower than in rats from groups 1 and 2, as well as lower, albeit insignificantly, from the respective baseline value (Table 4).

### IL-6 in urine

The most pronounced, 40-fold increase in urinary IL-6 was observed at the 14<sup>th</sup> day of the experiment in ani-

mals from group 3, i.e. those infected with the highest dose of *E. coli*. In group 2, the urinary concentration of IL-6 at day 14 was four times higher than those at the baseline. Furthermore, statistically significant differences were observed in the urinary concentrations of IL-6 in rats from groups 1, 2 and 3 (Table 5).

### 24-h intake of water

No significant intergroup differences in 24-h intake of water were observed at days 7, 14 and 21. Irrespective of the group, mean intake of water amounted to 18-20.5 ml per animal per day.

**Table 3.** FENa (the result given in %)

Day	Group 1. 10 <sup>5</sup> cfu/ml	Group 2. 10 <sup>7</sup> cfu/ml	Group 3. 10 <sup>9</sup> cfu/ml	ANOVA
0	0.19±0.07	0.19±0.7	0,19±0.07	
7	0.09±0.04 #p=0.002	0.18±0.07 *p=0.021	0.07±0.05 #p=0.001 **p=0,01	p<0.001
14	0.13±0.09	0.47±0.34 #p=0.002 *p=0.001	0.13±0.05 **p=0.003	p<0.001
21	0.1±0.05	0.19±0.17	0,09±0.04 #p=0.048	p=0.03

# - statistically significant differences between the study group and the control group – HSD Tukey Test

\* - statistically significant differences between the study group and the group 1 on the corresponding day – HSD Tukey Test

\*\* - statistically significant differences between the study group and the group 2 on the corresponding day – HSD Tukey Test

**Table 4.** FEUrea ( the result given in %)

Day	Group 1. 10 <sup>5</sup> cfu/ml	Group 2. 10 <sup>7</sup> cfu/ml	Group 3. 10 <sup>9</sup> cfu/ml	ANOVA
0	49.29±16.05	49.29±16.05	49.29±16.05	
7	55.14±12.24	61.12±7.4	48±12.92	NS
14	65.57±11.94	63.12±17	40.17±3.97 *p=0.017 **p=0.029	p=0.005
21	55.43±10.64	53.12±17.88	51.33±15.31	NS

NS – not statistically significant

\* - statistically significant differences between the study group and the group 1 on the corresponding day – HSD Tukey Test

\*\* - statistically significant differences between the study group and the group 2 on the corresponding day – HSD Tukey Test

**Table 5.** IL-6 in urine ( the result given in pg/24h)

Day	Group 1. 10 <sup>5</sup> cfu/ml	Group 2. 10 <sup>7</sup> cfu/ml	Group 3. 10 <sup>9</sup> cfu/ml	ANOVA
0	13.79±1.63	13.79±1.63	13.79±1.63	
7	15.53±0.42	16.58±2.15	23.18±1.28 #p<0.001 * p<0.001 **p=0.001	p<0.001
14	21.85±6.51	55.7±1.31 #p<0.001 *p=0.001	554.25±19.25 #p<0.001 *p<0.001 **p<0.001	p<0.001
21	17.53±4.07	15±2.6	17.87±0.4 #p=0.048	p=0.046

# - statistically significant differences between the study group and the control group – HSD Tukey Test

\* - statistically significant differences between the study group and the group 1 on the corresponding day – HSD Tukey Test

\*\* - statistically significant differences between the study group and the group 2 on the corresponding day – HSD Tukey Test



## Body temperature

None of the examined animals presented with elevated body temperature at day 7, 14 and 21.

## DISCUSSION

Urine samples obtained at various stages of the experiment were examined microbiologically in order to verify if transurethral inoculation with *E. coli* resulted in the development of UTI in all the studied groups. In humans, the diagnosis of UTI is confirmed isolating bacteria from routinely collected biological material, urine. The bacterial count in urine is verified against the strict diagnostic criteria of UTI [13,38]. Due to the lack of standardized methodology of urine collection for microbiological purposes and specific diagnostic criteria for UTI in laboratory animals, we were only able to confirm the presence of bacteria in the urinary tract. The fact that no statistically significant differences in bacterial counts were found between animals inoculated with various doses of *E. coli* likely reflected the lack of standardized methodology for urine collection. Therefore, markers of renal damage were determined to confirm the activity and severity of urinary tract inflammation.

Histopathologic examination documented inflammatory lesions in renal parenchyma of all animals with experimentally induced pyelonephritis. The chronic character of lesions observed at 21 days of UTI induction implies that an acute process took place at early stages of the experiment.

Further analysis, including diagnostic parameters of AKI recommended by KDIGO, i.e. diuresis and CreaS, demonstrated that renal filtering function was substantially impaired solely in the groups inoculated with intermediate or high dose of *E. coli* (groups 2 and 3). At 7 and 14 days post-inoculation, values of these parameters in the two groups differed significantly from those determined at the baseline, which implies that an acute phase of the kidney injury developed earlier than suggested by histopathologic findings. This hypothesis was further supported by the data on urinary concentration of IL-6 (Table 5).

Urinary IL-6 is a marker of inflammatory activity within the urinary tract [4,25]. Moreover, it is used as an early marker of AKI and a prognostic factor for permanent renal damage [5,16,19]. Rugo et al. analyzed urinary excretion of IL-6 during the course of pyelonephritis. They observed that urinary concentrations of IL-6 were the highest at 6-12 h after activation of the inflammatory process, and then decreased gradually down to their baseline level [30]. In the hereby presented experiment, we did not measure urinary IL-6 at early stages of UTI. However, urinary levels of IL-6 after inoculation with both intermediate and high dose of *E. coli* ( $10^7$  cfu/ml and  $10^9$  cfu/ml for group 2 and 3, respectively), were the highest at day 14. This implies that both these groups

were at risk of AKI, and the latter was more evident in rats from group 3, i.e. those inoculated with  $10^9$  cfu/ml of uropathogenic *E. coli*.

Bacterial colonization and the resultant inflammation of renal parenchyma were reflected by a significant decrease in the number of medullary nephrons in animals from group 2 (inoculated with  $10^7$  cfu/ml of *E. coli*), as well as by the loss of both cortical and medullary nephrons in rats from group 3 (inoculated with  $10^9$  cfu/ml). These lesions were associated with impaired diuresis, an increase in CreaS level and a decrease in ClCr, a marker of GFR. At 14 and 21 days after induction of pyelonephritis, 24-h urine output in animals from group 2 ( $10^7$  cfu/ml) and 3 ( $10^9$  cfu/ml) was ~ 50% and ~ 40% lower than at the baseline. Since reduced or sustained urine production is a diagnostic criterion for AKI, it can be hypothesized that rats exposed to intermediate or high dose of *E. coli* ( $10^7$  cfu/ml or  $10^9$  cfu/ml, respectively) developed this condition between 7 and 21 days of the experiment (Figure 2, Table 2).

Also an increase in CreaS by 40% at day 7 in group 3 (animals inoculated with  $10^9$  cfu/ml of *E. coli*) and by 35% at day 14 in group 2 (rats exposed to  $10^7$  cfu/ml of the bacteria) points to the deterioration of renal function. A similar 30-40% increase in serum creatinine between 3 and 14 day of the experiment was reported by Malekinejad et al. after the administration of  $10^6$ - $10^7$  cfu/ml of *E. coli* to renal parenchyma [21]. Markedly more evident, a nearly five-fold increase in CreaS was documented by Sener et al., who induced interstitial pyelonephritis by injecting  $10^{10}$  cfu/ml of *E. coli* [32]. In our experiment, the increase in CreaS was reflected by a decrease in GFR, by 48% at day 14 in animals from group 2 (inoculated with  $10^7$  cfu/ml) and by 43% and 52% at days 7 and 14, respectively, in rats from group 3, i.e. those exposed to *E. coli* at  $10^7$  cfu/ml. A substantially greater reduction of GFR, by even 71%, was previously reported by Heyman et al. in the model AKI induced by the administration of a contrast agent [11]. With no doubt, the degree of renal function impairment, expressed by an increase in serum creatinine and GFR reduction, depends on the route via which the triggering factor was administered (interstitial, transurethral), as well as on the type (bacteria, contrast agent) and dose of the latter. This is likely the reason why an increase in serum creatinine in rats inoculated with the intermediate dose of *E. coli* was observed later than in the group exposed to the larger inoculum. Since an increase in CreaS is another diagnostic criterion of AKI [29], our findings imply that transurethral administration of *E. coli* at  $10^7$  cfu/ml and  $10^9$  cfu/ml induced AKI between 7 and 21 days post-inoculation, and functional impairment of the kidneys was proportional to the inoculum size.

Electrolyte concentrations during the course of AKI are important laboratory parameters not only in view of potentially harmful consequences of dyselectrolytemia, but also due to the fact that their changes may

be specific for the cause of kidney damage [33]. FENa is an example of a parameter used in the differential diagnosis of various AKI forms [10]. According to literature, a decrease in FENa is specific for the prerenal etiology of this condition, whereas an increase in this parameter points to renal abnormalities as a cause of AKI [8,16]. Our findings suggest that in rats from group 3, AKI not only resulted from renal damage but had also a prerenal component, probably as a consequence of systemic bacterial infection. The decrease in FENa observed in animals from group 3 ( $10^9$  cfu/ml) was not associated with hypovolemia resulting from fever or reduced water intake. Consequently, the prerenal etiology of AKI was probably related to the dilation of the vascular bed by bacterial toxins and other systemic effects of vasodilating agents released during the course of infection.

Rats exposed to the highest dose of *E. coli* ( $10^9$  cfu/ml) showed a decrease in FENa at each stage of the experiment, by 63%, 32% and 53% at days 7, 14 and 21, respectively. An opposite phenomenon was observed in the group exposed to the intermediate dose of the bacteria ( $10^7$  cfu/ml), in which FENa at day 14 corresponded to 147% of the baseline value. These findings point to a renal etiology of AKI in group 2 ( $10^7$  cfu/ml) and a prerenal etiology of this condition in group 3 ( $10^9$  cfu/ml). Langenberg et al. analyzed a model AKI induced by the administration of *E. coli* ( $3.9 \times 10^9$  cfu/ml) to the bloodstream, and observed a 92% reduction of FENa [17]. Probably, this relatively greater decrease in FENa reflected not only the different route of administering bacterial inoculum and resultant greater systemic *E. coli* count than in our study, but also the absence of a renal component of AKI. Another argument for the prerenal origin of kidney injuries in rats from group 3 ( $10^9$  cfu/ml) seems to be a decrease in FEUrea, another marker of AKI etiology [15]. Animals inoculated with the highest dose of *E. coli* were the only group presenting with lower FEUrea values, as compared to both the baseline and to other rats. However, none of these differences were statistically significant, which implies that this parameter is of secondary importance and more attention should be paid to FENa. In this study, we did not compare empirical FENa and FEUrea values to any reference or cutoff limits, but analyzed their relative changes from the baseline. A relative decrease in both parameters observed in group 3 ( $10^9$  cfu/ml) points to a likely prerenal etiology of the kidney injury in animals exposed to the highest dose of *E. coli* (Tables 3 and 4).

We found an association between the size of *E. coli* inoculum and the effects of its administration. Inoculation with the lowest dose of *E. coli* ( $10^5$  cfu/ml) resulted in the least pronounced impairment of renal function, but even in this group histopathologic examination reveal bacterial colonization of kidney parenchyma. The larger the size of *E. coli* inoculum, the more evident the alterations of analyzed parameters and the greater the degree of renal inflammation. Our findings imply that, depending on the inoculum size, transurethral administration

of *E. coli* may result either in the development of renal AKI or in systemic infection. This observation seems to be vital from a clinical perspective, suggesting that even a patient with asymptomatic bacteriuria, who does not qualify for antibacterial treatment in line with current therapeutic guidelines, may develop tubulointerstitial nephritis and thus be at increased risk for acute or chronic renal damage. Consequently, all patients with asymptomatic bacteriuria should be optimally subjected to molecular analyses to identify genotypic profile of virulence factors expressed by their *E. coli* isolates, and antibiotic therapy should be implemented whenever a strain expressing fimbriae responsible for colonization of structures other than those present in urinary bladder (type II fimbriae – P, S involved in colonization of renal structures and vascular endothelium) was detected [20]. According to literature, 30-50% of patients with lower urinary tract infections may also present with asymptomatic pyelonephritis [6]. Thus, phenotyping seems to be the most reasonable diagnostic option, especially in individuals with other risk factors of acute kidney injury, such as older age, diabetes mellitus, chronic kidney disease, sepsis and circulatory failure.

The data presented in this study imply that the experimental model for AKI is dose-dependent. Irrespective of the inoculum size, administration of *E. coli* isolate being capable of colonizing upper urinary tract (i.e. expressing type II fimbriae) always led to UTI and resultant pyelonephritis. However, the amount of inoculated bacteria determined the severity of the infection and the extent of inflammation in a dose-dependent manner. While transurethral inoculation with highly virulent *E. coli* at both  $10^7$  and  $10^9$  cfu/ml resulted in pyelonephritis, the lower dose induced solely an isolated AKI, whereas inoculation with the higher dose resulted not only with ascending pyelonephritis, but also with AKI of prerenal etiology, probably linked to bacteremia and urosepsis.

To the best of our knowledge, none of the previous studies analyzed the relationship between the size of *E. coli* inoculum used to induce pyelonephritis and biochemical indicators of renal function. As stated above, transurethral administration of *E. coli*, regardless of the inoculum size, will always result in renal inflammation. Inoculation with a higher dose of *E. coli*,  $10^7$  cfu/ml or  $10^9$  cfu/ml, may pose a risk of AKI. According to literature, the later condition may develop within a few hours to several days after the administration of a triggering factor [22,26]. Our findings presented in this study, specifically the changes in the markers of AKI (diuresis, CreaS), suggest that the duration of the latency period correlates inversely with bacterial inoculum size. Future studies, involving microbiological cultures of the blood, determination of other markers of kidney damage and monitoring of renal function at shorter time intervals, should explain the role of systemic bacterial infection in AKI development, the timing of functional renal deterioration and the size of inoculum necessary to develop fully symptomatic kidney damage.



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